

## PATENT COOPERATION TREATY

## PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY  
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

REC'D 23 MAY 2006



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Applicant's or agent's file reference PCT-2757	<b>FOR FURTHER ACTION</b> See Form PCT/IPEA/416	
International application No. <b>PCT/KR2005/000234</b>	International filing date(day/month/year) <b>27 JANUARY 2005 (27.01.2005)</b>	Priority date (day/month/year) 30 JANUARY 2004 (30.01.2004)
International Patent Classification (IPC) or national classification and IPC  <b>C12N 9/24(2006.01)i, C12N 9/30(2006.01)i, C12N 15/56(2006.01)i, C12N 15/63(2006.01)i, C12N 9/00(2006.01)i</b>		
Applicant  <b>LIFENZA CO., LTD. et al</b>		

- This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
- This REPORT consists of a total of 4 sheets, including this cover sheet.
- This report is also accompanied by ANNEXES, comprising:
  - ☒ (sent to the applicant and to the International Bureau) a total of 4 sheets, as follows:
    - ☒ sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
    - ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
  - ☐ (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) \_\_\_\_\_ containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box relating to Sequence Listing (see Section 802 of the Administrative Instructions).

- This report contains indications relating to the following items:
  - ☒ Box No. I Basis of the report
  - ☐ Box No. II Priority
  - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
  - ☐ Box No. IV Lack of unity of invention
  - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
  - ☐ Box No. VI Certain documents cited
  - ☐ Box No. VII Certain defects in the international application
  - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand  <b>24 AUGUST 2005 (24.08.2005)</b>	Date of completion of this report  16 MAY 2006 (16.05.2006)
Name and mailing address of the IPEA/KR  Korean Intellectual Property Office 920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	Authorized officer  CHO, YOUNG GYUN  Telephone No. 82-42-481-8132 

# INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

PCT/KR2005/000234

## Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on translations from the original language into the following language \_\_\_\_\_ which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3 and 23.1(b))

☐ publication of the international application (under Rule 12.4)

☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

☐ the international application as originally filed/furnished

☒ the description:

pages 1-6, 8-18 as originally filed/furnished

pages\* 7 received by this Authority on 07/04/2006

pages\* \_\_\_\_\_ received by this Authority on \_\_\_\_\_

☒ the claims:

pages 20 as originally filed/furnished

pages\* \_\_\_\_\_ as amended (together with any statement) under Article 19

pages\* 19 received by this Authority on 07/04/2006

pages\* \_\_\_\_\_ received by this Authority on \_\_\_\_\_

☒ the drawings:

pages 1/6-6/6 as originally filed/furnished

pages\* \_\_\_\_\_ received by this Authority on \_\_\_\_\_

pages\* \_\_\_\_\_ received by this Authority on \_\_\_\_\_

☒ the sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages \_\_\_\_\_

☐ the claims, Nos. \_\_\_\_\_

☐ the drawings, sheets \_\_\_\_\_

☐ the sequence listing (*specify*): \_\_\_\_\_

☐ any table(s) related to sequence listing (*specify*): \_\_\_\_\_

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages \_\_\_\_\_

☐ the claims, Nos. \_\_\_\_\_

☐ the drawings, sheets \_\_\_\_\_

☐ the sequence listing (*specify*): \_\_\_\_\_

☐ any table(s) related to sequence listing (*specify*): \_\_\_\_\_

\* If item 4 applies, some or all of those sheets may be marked "superseded."

## INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

PCT/KR2005/000234

**Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

## 1. Statement

Novelty (N)	Claims	1-10	YES
	Claims	None	NO
Inventive step (IS)	Claims	1-10	YES
	Claims	None	NO
Industrial applicability (IA)	Claims	1-10	YES
	Claims	None	NO

## 2. Citations and explanations (Rule 70.7)

The following documents have been considered for the purpose of this report:

D1: WO 2003/018790 A1 (LIFENZA CO., LTD.) 6 MARCH 2003

D2: WO 2001/066570 A1 (KIM et al.) 13 SEPTEMBER 2001

D3: J. Microbiol. Biotechnol., Vol. 9(3), pp. 260-264 (1999)

D4: Biosci. Biotechnol. Biochem., Vol. 64(2), pp. 223-228 (2000)

The present invention relates to an enzyme, having the amino acid sequence of SEQ. ID. NO:1, with the activity of hydrolyzing dextran, starch, mutan, inulin and levan; a gene (SEQ. ID. NO:2) encoding said enzyme; a transformed cell expressing said gene; a method of producing said enzyme; and a composition for the dextran removal and the plaque elimination.

D1-D4 disclose the DEXAMase (dextranase and amylase), having antiplaque and anticaries activities, having dextranase and amylase activities simultaneously and degrading insoluble glucans, from *Lipomyces starkeyi* KSM 22; a preparation method of DEXAMase; and an oral composition comprising the same.

However, none of the prior art documents disclose the amino acid sequence of the enzyme (SEQ. ID. NO:1) and the nucleotide sequence of gene (SEQ. ID. NO:2) encoding the enzyme, and said enzyme in this invention cannot be derived in an obvious manner from the prior art documents.

Therefore, claims 1-10 meet the requirements of novelty, inventive step and industrial applicability under PCT Article 33(2)-(4).

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

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Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

a. type of material



a sequence listing



table(s) related to the sequence listing

b. format of material



on paper



in electronic form

c. time of filing/furnishing



contained in the international application as filed



filed together with the international application in electronic form



furnished subsequently to this Authority for the purposes of search and/or examination



received by this Authority as an amendment\* on 07/04/2006

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed of furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

(corresponding to U.S. Pat. No. 6,485,953 dated Nov. 26, 2002) which relates to a DXAMase enzyme capable of hydrolyzing both dextran and starch, a microorganism producing the enzyme (identified as *Lipomyces starkeyi* KFCC-11077), and a  
5 composition comprising the enzyme.

The enzyme expressed from the gene (*lsd1*) of the present invention is capable of hydrolyzing starch and mutan (insoluble glucan) as well as dextran. Also, the glycanase according to the present invention is found to degrade dextran  
10 mainly into glucose, isomaltose and isomaltotriose, with the concurrent production of smaller amounts of branched pentaoses and hexaoses.

Both levan- and inulin-type fructans, which are constituents of dental plaque, can be degraded by the  
15 glycanase according to the present invention.

Accordingly, effective degradation of glucans, whether soluble or insoluble, can be achieved by the glycanase of the present invention. As it can prevent the formation of plaque and remove previously formed plaque by inhibiting the  
20 colonization of bacteria and the aggregation of glucans, the glycanase is useful in preventing tooth cavities. It is inferred that the glycanase has the ability to remain on the teeth as demonstrated by a test for whether or not the enzyme binds to hydroxyapatite which is similar to tooth enamel  
25 components.

Also, the present invention is concerned with a novel microorganism carrying a gene encoding the glycanase. The microorganism, a *Saccharomyces cerevisiae* pYLSd1, was

## WHAT IS CLAIMED IS:

1. A protein, comprising an amino acid sequence of SEQ. ID. No. 1, which has the activity of hydrolyzing dextran, starch, mutan, inulin and levan, a derivative thereof, or a fragment thereof.
2. A gene of SEQ. ID. No. 2, encoding the protein, the derivative, or the fragment of claim 1, a derivative thereof, or a fragment thereof.
3. A transformed cell, expressing the gene, the derivative, or the fragment of claim 2.
4. The transformed cell as defined in claim 3, wherein the cell is prokaryotic or eukaryotic.
5. The transformed cell as defined in claim 3 or 4, wherein the cell is *Saccharomyces cerevisiae* pYLSD1 deposited on Dec. 24, 2003, with the accession number KCTC 10574BP.
6. A method of producing an enzyme having activity of hydrolyzing dextran, starch, mutan, inulin and levan, comprising:
  - culturing the cell of claim 3;
  - expressing the enzyme in the cultured cell; and
  - purifying the expressed enzyme.

## 【Sequence Listing】

<110> Lifenza Co., Ltd.

<120> PROTEIN WITH ACTIVITY OF HYDROLYZING DEXTRAN, STARCH, MUTAN,  
 5 INULIN AND LEVANN, GENE ENCODING THE SAME, CELL EXPRESSING THE  
 SAME, AND PRODUCTION METHOD THEREOF

<150> KR2004-0006185  
 <151> 2004-01-30

10 <160> 4

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15 <210> 1  
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 <212> PRT  
 <213> Artificial Sequence

20 <220>  
 <223> *Saccharomyces cerevisiae* pYLSD1

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 1 5 10 15

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 20 25 30

30 Ala Ala Val Leu Pro Arg Asp Asn Arg Thr Val Cys Gly Ser Gln Leu  
 35 40 45

Cys Thr Trp Trp His Asp Ser Gly Glu Ile Asn Thr Gly Thr Pro Val  
 35 50 55 60

Gln Ala Gly Asn Val Arg Gln Ser Arg Lys Tyr Ser Val His Val Ser

AMENDED SHEET (ART. 34)
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	Thr	Gly	Ile	Ser	Ile	Asp	Asn	Leu	His	Val	Ile	His	Thr	Arg	Tyr	Phe
	465					470							475			480
5	Lys	Ser	Glu	Thr	Val	Val	Pro	Ser	Ala	Ile	Ile	Gly	Ala	Ser	Pro	Phe
					485					490					495	
	Tyr	Ala	Ser	Gly	Met	Thr	Val	Asp	Pro	Ser	Glu	Ser	Ile	Ser	Met	Thr
				500					505					510		
10	Ile	Ser	Asn	Val	Val	Cys	Glu	Gly	Leu	Cys	Pro	Ser	Leu	Phe	Arg	Ile
			515						520					525		
	Thr	Pro	Leu	Gln	Ser	Tyr	Asn	Asn	Leu	Val	Val	Lys	Asn	Val	Ala	Phe
	530							535					540			
15	Pro	Asp	Gly	Leu	Gln	Thr	Asn	Pro	Ile	Gly	Ile	Gly	Glu	Ser	Ile	Ile
	545						550					555			560	
	Pro	Ala	Ala	Ser	Gly	Cys	Thr	Met	Asp	Leu	Glu	Ile	Thr	Asn	Trp	Thr
20					565					570					575	
	Val	Lys	Gly	Gln	Lys	Val	Thr	Met	Gln	Asn	Phe	Gln	Ser	Gly	Ser	Leu
				580						585					590	
25	Gly	Gln	Phe	Asp	Ile	Asp	Gly	Ser	Tyr	Trp	Gly	Gln	Trp	Ser	Ile	Asn
		595						600					605			

30

<210> 2  
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<212> DNA  
<213> Artificial Sequence

35

<220>  
<223> *Saccharomyces cerevisiae* pYLS1

AMENDED SHEET (ART. 34)